Verotoxigenic *Escherichia coli* (VTEC): A major public health threat in Canada

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BACKGROUND: Verotoxigenic *Escherichia coli* (VTEC) was first described in Canada during the 1980s as an emerging food-borne disease in association with morbidity and mortality in outbreaks of hemorrhagic colitis caused by *E coli* O157:H7.
OBJECTIVE: To describe the surveillance activities and epidemiological laboratory markers of VTEC that are used at the National Laboratory for Enteric Pathogens (NLEP) to investigate sporadic cases and outbreaks of *E coli* O157:H7 and non-O157 VTEC in Canada.
METHODS: Passive surveillance was conducted by obtaining data on laboratory confirmed cases of VTEC from the Provincial Laboratories of Public Health across Canada. The laboratory epidemiological markers generated for isolates of VTEC included biotyping, serotyping, phage typing, toxin detection and characterization, and molecular typing using pulsed-field gel electrophoresis.
RESULTS: Major outbreaks of VTEC O157:H7 disease have been associated with ground beef, unpasteurized apple juice, salami and untreated water. In 1999 and 2000, a total of 46 outbreaks of *E coli* O157:H7 disease were investigated. Among those, one outbreak was associated with contact at a petting zoo and a second with the consumption of salami. An outbreak in 2000 in Ontario was associated with water and resulted in more than 1000 cases of human illness, with six deaths. The NLEP has also identified more than 100 non-O157 VTEC serotypes from cattle and meat products. At least 23 VTEC serotypes found in humans were also identical to those found in cattle and meat products.
CONCLUSIONS: The laboratory-based information that is generated is used to define the incidence, sources of infection, risk factors, trends, distribution and transmission of VTEC to humans from food, water and animal sources. Prevention and control of outbreaks are high-priority health concerns.

Key Words: *Epidemiological markers; Outbreaks; Verotoxigenic* Escherichia coli *(VTEC)*

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Escherichia coli producteur de vérocytotoxine (ECVP) : Une menace pour la santé publique canadienne

HISTORIQUE : L'infection à ECPV (*Escherichia coli* producteur de vérocytotoxine) a d'abord été décrite au Canada au cours des années 1980 en tant que nouvelle maladie d'origine alimentaire et a été associée à la morbidité et à la mortalité lors d'éclosions de colite hémorragique causée par *E. coli* O 157:H7.

OBJECTIF : Décrire les activités de surveillance et les marqueurs épidémiologiques d'ECPV en laboratoire qui sont utilisés au Laboratoire national des agents pathogènes entériques afin d'étudier les cas sporadiques et les éclosions d'infections à *E. coli* O 157:H7 ou d'ECPV non-O 157 au Canada.

MÉTHODES : Une surveillance passive a été effectuée par l'obtention de données sur des cas d'ECPV confirmés en laboratoire à partir des laboratoires provinciaux de santé publique de tout le Canada. Les marqueurs épidémiologiques générés en laboratoire pour les isolats d'ECPV incluaient le biotypage, le sérotypage, la détermination des types, le dépistage des toxines et la caractérisation de même que le typage moléculaire à l'aide d'électrophorèse sur gel à champ pulsé.

RÉSULTATS : Les principales éclosions de maladie à ECPV O157:H7 ont été associées au bœuf haché, au jus de pomme non pasteurisé, au salami et à l'eau non traitée. En 1999 et 2000, en tout, 46 éclosions de *E. coli* O157:H7 ont été analysées. Parmi celles-ci, l'une a été associée à un contact avec un animal et la deuxième à la consommation de salami. En l'an 2000, en Ontario, une éclosion a été associée à l'eau et a entraîné plus de 1 000 cas de maladie dont six décès chez l'être humain. Le Laboratoire national des agents pathogènes entériques a aussi identifié plus de 100 sérotypes d'ECPV non-O157:H7 dans des élevages et dans des produits de boucherie. Au moins 23 sérotypes d'ECPV ont été découverts chez l'être humain. Ils étaient identiques à ceux que l'on a isolés dans le bétail et les animaux de boucherie.

CONCLUSION : L'information fondée sur les analyses de laboratoire est utilisée pour définir l'incidence, les sources d'infection, les facteurs de risque. Les tendances, la distribution et la transmission d'ECPV à l'être humain à partir des aliments, de l'eau et des animaux. La prévention et la lutte contre les éclosions sont d'importantes priorités pour la santé publique.

Escherichia coli O157:H7 has emerged as a major cause of both outbreaks and sporadic cases of human diarrheal disease in North America and throughout the world (1-3). Major outbreaks have occurred worldwide, and 70% to 80% of sporadic cases of classic hemolytic uremic syndrome (HUS) reported in Canada, the United Kingdom, Germany, Belgium, the Netherlands and Japan have been associated with *E coli* O157:H7 infections (4). The United States Centers for Disease Control and Prevention estimate that, in the United States alone, this agent causes approximately 73,000 illnesses and more than 60 deaths/year.

Verotoxigenic *E coli* (VTEC) or Shiga-toxigenic *E coli*, including O157:H7 and other non-O157 serogroups, produce verotoxins (VTs) (verocytotoxins) that result in human disease. These toxins produce profound cytopathic effects in vero cells, and they show a high degree of homology to the Shiga-toxin (Stx) of *Shigella dysenteriae* type 1 (5). Clinical symptoms may include bloody diarrhea and hemorrhagic colitis, along with complications associated with HUS, acute and chronic kidney disease, thrombotic thrombocytopenic purpura (TTP), neurological sequelae and death (4,6-12). In outbreaks reported between 1982 and 1983, 23% of patients were hospitalized, 6% developed HUS or TTP and 1.2% died (5). In 1982, two outbreaks of *E coli* disease occurred in the United States, followed in the same year by a further outbreak in Canada. It was this latter outbreak that led to the recognition of a new pathogenic serotype, *E coli* O157:H7 (2,9,13). In 1985, Canada experienced one of its worst outbreaks of *E coli* O157:H7 in a nursing home, which resulted in the deaths of 17 elderly residents (6). However, it was the spectacular outbreak caused by undercooked hamburgers obtained at a fast-food restaurant chain in the United States in 1993 that catapulted the pathogen and the condition that it caused into the limelight (2) and earned it the popular name 'hamburger disease'.

E coli O157:H7 has been isolated from new sources and in increasing numbers as a cause of human infection (3,6,7). Indeed, a number of food sources have emerged as vehicles for transmission and, as a consequence, these pose major health threats to populations worldwide. Major outbreaks of VTEC O157:H7 have been associated with ground beef (14-16), unpasteurized milk (7), unpasteurized apple juice (17), salami (18), alfalfa sprouts (19), lettuce (7) and untreated water (7). This wide distribution of VTEC in foods and contaminated water, along with VTEC's ability to cause severe human diarrheal disease, make the implementation of proper food handling practices and public education on food safety critical to reducing the disease burden in the general population.

Surveillance for pathogens and the early identification of outbreaks are also critical for reducing the incidence of foodborne disease. The Canadian National Laboratory for Enteric Pathogens (NLEP) uses surveillance and laboratory-based epidemiological markers specific for bacterial isolates to track human infections, and to identify and characterize outbreaks (20). *E coli* O157:H7 and isolates of non-O157 VTEC are confirmed using biochemical and serological identification techniques. Isolates are also examined for the production of VTs and their associated virulence genes. Further subtyping of VTEC O157 using phage typing and molecular typing provides key epidemiological markers for tracing sources of bacteria responsible for human disease, for trace back analysis and for supporting food or product recalls. Phage typing of *E coli* O157:H7 allows the differentiation of isolates into 88 phage types. VT genotypes and other VTEC and enterohemorrhagic *E coli* (EHEC) virulence factors (eg, *eae* and hemolysin genes) are determined using a combination of polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (RFLP) techniques. Further strain discrimination is provided by pulsed-field gel electrophoresis (PFGE). The use of a combination of phage typing, VT genotyping and PFGE provides excellent strain discrimination for outbreak investigations and surveillance. Trends in the epidemiology of VTEC infection in Canada have been reviewed (21). The purpose of the present article is to provide an analysis of the bacterial strains responsible for human disease in Canada.

MATERIALS AND METHODS

Strains

Human isolates of VTEC were submitted for identification by the Provincial Laboratories of Public Health and from the territories across Canada. Strains were provided to the NLEP as part of a passive surveillance system. The isolates analyzed, therefore, represent a subset of isolates associated with human illness in Canada.

Isolates were characterized in a number of ways, including: the use of biochemical assays to identify VTEC O157:H7 (biotyping); the assessment of somatic “O” and flagellar “H” antigens of both *E coli* O157:H7 and non-O157:H7 VTEC (serotyping); phage typing; molecular typing; and toxin detection.

Biotyping

Sorbitol-MacConkey Agar (SMAC) is the most widely used medium for the isolation of O157 VTEC (Table 1). Strains of *E coli* O157:H7 do not ferment sorbitol at 24 h; however, some strains of *E coli* O157:H7 and O157:H–, as well as non-O157 VTEC, are sorbitol positive at 24 h and are, therefore, not detected by SMAC.

Biochemical assays and the reaction profiles used to identify *E coli* O157 include: positive in indole, lysine decarboxylase, glucose (with gas) and *o*-nitro-phenyl-β-galactosidase; negative for urea, sorbitol and sodium malonate; variable in methyl red and negative in Voges-Proskauer and acid or alkaline over acid in triple sugar iron agar. Rainbow agar (Biolog Inc, USA) O157 has also been used with variable success for the isolation and differentiation of VTEC from non-VTEC isolates. Colonies of *E coli* O157:H7 strains show a unique and distinctive black colour, while those of non-VTEC strains are blue or purple and most nontoxigenic isolates appear reddish in color.

Beta-D-glucuronidase is an enzyme that can cleave a 4-methylumbelliferyl-β-D-glucuronide (MUG) reagent (22,23). Positive β-D-glucuronidase *E coli* cleave the MUG reagent and the end product is visible as fluorescence using ultraviolet at a wavelength of 365 nm. Approximately 96% of *E coli* are β-D-glucuronidase-positive, but the majority of *E coli* O157:H7 and O157:H– strains are MUG-negative (Table 1).

Serotyping

Serotyping of *E coli* was performed by slide agglutination with monoclonal/latex antisera using nonsorbitol fermenting *E coli*. Confirmatory tests for *E coli* O157, non-O157 VTEC, H7 and other non-H7 antigens were performed by tube agglutination using heat-treated O antigens and formalin-treated H antigens (24).

TABLE 1
Phenotypic profiles of *Escherichia coli* O157 associated with verotoxin (VT)

Antigenic structure	VT	Sorbitol	β-D-glucuronidase
O157:H7*	+	–	–
O157:H7	+	–	+
O157:H7	+	+	+
O157:H–*	+	+	+
O157:H7*	–	–	–
O157:H–*	–	+	+
O157:H–*	–	–	+
O157:H–*	–	+	–

**Observed among Canadian isolates*

Phage typing

E coli O157:H7 strains isolated from humans, the environment and animals associated with outbreaks were received at the NLEP, Health Canada, Winnipeg, Manitoba. All strains were stored at –70°C and working cultures were maintained on nutrient agar slopes (Oxoid Ltd, England). Phage typing of *E coli* O157:H7 was performed using published techniques (25,26). Briefly, strains were plated on nutrient agar and incubated for 18 h at 37°C. A single smooth colony was inoculated into 4.5 mL of Difco (Becton, Dickinson and Company, USA) phage broth (pH 6.8) and incubated at 37°C for 2.5 h in a shaking water bath. The bacterial cultures were inoculated by flooding a Difco phage agar plate to form a smooth lawn. A panel of 16 phages at their routine test dilution was spotted on the bacterial lawn and the plates were allowed to dry. The plates were incubated at 37°C for 18 h before examination for lytic patterns. An enhanced scheme to subdivide *E coli* phage type 14 into four subgroups by using four additional phages was introduced in 2000.

PFGE and VT typing

PFGE was performed using methods and standards developed in-house for the first few years of the study. However, in 1998, the NLEP adopted the method of Barrett et al (14), which was modified to correspond with the PulseNet standardized method (27). In brief, bacterial cell concentrations were adjusted to give an optical density reading of 0.68 to 0.72. Plugs were made, washed and digested according to the standard protocol. Electrophoresis was carried out in 1% agarose gels using Seakem Gold agarose (Mandel Scientific Co, Ltd, Canada). The PFGE buffer used was 0.5 × Tris-borate-ethylenediamine-tetraacetic acid (TBE) made from 5× TBE buffer concentrate (Sigma-Aldrich Canada, Ltd, Canada). Switch times were 2.2 to 54.2 s and the run time was 20 h.

Gels were run at a temperature of 14°C and a voltage of 6 V/cm in a Chef-DR III PFGE unit (Bio-Rad, Canada). Gels were stained in 0.5 µg/mL ethidium bromide and the DNA was visualized with an Alpha Imager 2000 (Alpha

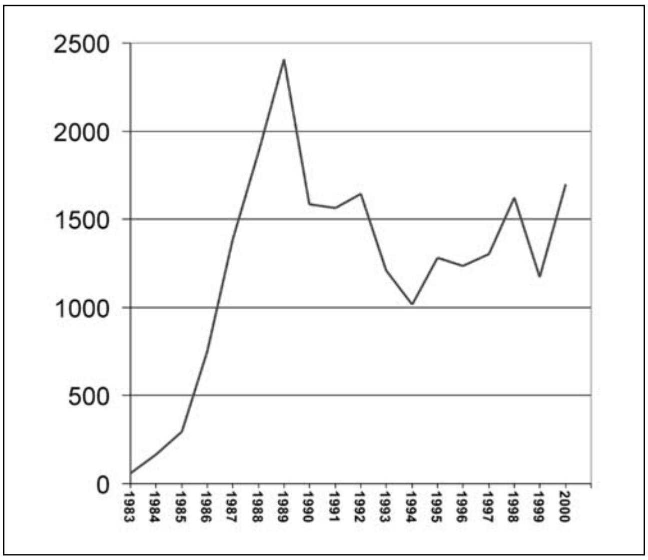

Figure 1) *Human laboratory confirmed cases of* Escherichia coli *O157:H7 in Canada between 1983 and 2000*

Innotech Corp, USA). All isolates tested were analyzed using the restriction enzyme Xba I, while selected isolates were additionally analyzed using Bln I as the second enzyme. Patterns were labeled using a designation formula that included: the organism (EC = *E coli*), the enzyme (XAI = Xba I; BNI = Bln I), and a unique identifier (.0001, .0002, etc.). Any pattern with one or more bands different was given a unique identification number. PFGE results were standardized and interpreted in accordance with the criteria described previously (27,28). In the absence of epidemiological evidence suggesting that isolates were part of an outbreak, isolates having patterns with one or more band differences were considered to be unrelated. If no epidemiological information was available and isolates with identical or very similar patterns appeared to be part of a cluster of cases, epidemiologists were contacted to determine whether an epidemiological investigation was warranted.

Detection of VTs

The vero cell cytotoxicity and neutralization assays for the detection of VTs produced by *E coli* strains were performed as described (29,30). Rabbit polyclonal antisera for neutralization were produced in the NLEP by immunization of rabbits with concentrated bacterial cell supernatants containing VT1 or VT2, followed by the boosting of rabbits with purified toxin until high titres of specific antibody were obtained. PCR assays for the detection of VT1 and VT2 and EHEC virulence factors were done according to previously published protocols (31,32). Detection of VT2 variant B subunit genes was conducted using the PCR-RFLP method of Tyler et al (33). This method was extended to describe the newly defined VT2 variant sequences (34).

RESULTS

The NLEP has used surveillance and epidemiological markers to track sources of infection associated with VTEC that cause outbreaks of human diarrheal disease. Although surveillance based on laboratory confirmed cases over the past 18 years has identified *E coli* O157:H7 as the most prevalent serotype responsible for human VTEC infections in Canada, it should be borne in mind that the use of commercial detection kits for O157:H7, and the relative ease and low cost of detecting this serotype in preference to others undoubtedly skews the distribution data considerably. In 1983, there were 59 reported laboratory confirmed cases of *E coli* O157:H7. The number of cases increased to a peak of 2407 in 1989, and ranged from 1014 to 1643 cases/year during the 1990s. In 1999, there were 1174 laboratory confirmed cases, and by 2000, the number had increased to 1700 (Figure 1). The reasons for this pattern of infection in the human population are not known, although suggestions have been made that they may be related to changes in climate, in the ecological niche of the organism, or in consumer awareness regarding risk factors for infection (21).

Canada experienced its first major outbreak of VTEC disease, defined as one in which there were five or more cases, in the province of Ontario in 1985. This outbreak originated in a nursing home, was associated with lunch foods prepared on site, and resulted in the deaths of 17 elderly residents (6). Since then, many outbreaks have been associated with a variety of sources of transmission (Table 2). Ground beef was once thought to be almost exclusively responsible for the transmission of the organism; however, over the period surveyed, laboratory and epidemiological outbreak investigations were successful in identifying many vehicles of infection responsible for outbreaks, and often, these proved to be sources other than meat. In 1998, a major outbreak associated with the consumption of salami occurred simultaneously in Ontario and British Columbia, causing 114 laboratory-confirmed cases (18). In the same year, an outbreak comprising 26 cases occurred in Ontario and was associated with the consumption of apple cider (17). In 1999, there were 25 reported cases in Ontario associated with a petting zoo where school children came into direct contact with goats and sheep. A second outbreak that year, associated with the consumption of salami, occurred in British Columbia, Alberta and Ontario, with 42 cases reported. Finally, in 2000, a major outbreak of *E coli* O157:H7 disease occurred in Ontario and was associated with contaminated drinking water. In all, there were 1346 reported cases of illness identified. Of these, 167 were confirmed as *E coli* O157:H7, 27 people developed HUS and six people died (35).

The NLEP has identified more than 48 VTEC serotypes causing human diarrheal disease (Table 3) and, with the Laboratory for Foodborne Zoonoses in Guelph, Ontario (36), more than 100 VTEC serotypes associated with cattle and meat sources (Table 4). Of these, 23 VTEC serotypes common to cattle and meat sources were also identified in humans (Table 4). A study of VTEC isolates received at the NLEP based on the geographical distribution of the most common VTEC serotypes associated with human disease in Canada includes: O157:H7, O26:H11, O128:H12,

TABLE 2
Major outbreaks of *Escherichia coli* O157 in Canada since 1985*

Year	Province	Isolations	Phage types	Verotoxin genotypes	Source
2000	Ontario	226	14	VT2	Water
2000	Nunavut	14	14a†	VT1 and VT2	Hamburger
2000	Quebec and Northwest Territories	25	14a	VT1 and VT2	Hamburger
2000	Nova Scotia	16	14	VT1 and VT2	Unknown‡
1999	Quebec	5	8	VT1 and VT2	Nursing home
1999	British Columbia, Alberta and Ontario	42	14	VT1 and VT2	Salami
1999	Manitoba	5	14	VT1 and VT2	Ground beef
1999	Ontario	25	8, 14, 27, 70	VT1 and VT2	Petting zoo
1998	Ontario	26	14	VT1 and VT2	Apple cider
1998	Ontario and British Columbia	115	2, 8, 10, 14	VT1 and VT2	Salami
1998	Nova Scotia	25	14	VT1 and VT2	Potato salad
1998	Alberta	12	14	VT1 and VT2	Daycare
1991	Northwest Territories and Manitoba	103§	32	VT1 and VT2	Unknown¶
1985	Ontario	70	2	VT1 and VT2	Lunch foods at a nursing home

**Only large outbreaks in which isolates were characterized and the vehicles of transmission were established are shown; †In 2000, phage type 14 pattern was subdivided with the use of additional phages; ‡Spinach was suspected as the vehicle of transmission; §An additional 49 isolates were made but not typed; ¶Ground beef was suspected as the vehicle of transmission*

O157:H–, O126:H8, O1:H7, O111:H–, O26:H–, O103:H2, O121:H19 and O113:H21 (Table 5). Among the isolates investigated, one from British Columbia was identified serologically as O181:H49, representing the newest serotype of VTEC. These VTEC serotypes were also characterized by the ability to produce various types of VT and these appeared as VT1, VT2 and VT2 variant human (VT2vh), in combination with other toxins or alone (13). For O157:H7 VTEC strains in Canada during the period of 1994 to 2000, the VT1+VT2 toxin type was found in 80.5% of all isolates tested. VT1 only was found in 1.3% of isolates, VT2 only in 8.7% of isolates and other VT negative and VT2vh toxins were present in 9.5% of isolates (Table 6).

Subtyping methods using phage typing and PFGE as molecular markers have been used successfully to investigate outbreaks and to trace the source of human infection (14). The phage typing scheme used at the NLEP for the differentiation of *E coli* O157:H7 was developed in Canada and recognizes 88 different phage types (PTs) (25,26). By 1996, PT 14 had emerged as the predominant PT in Canada. In 2000, this PT was subdivided into types 14 and 14a. Since then, both have been associated with numerous sporadic cases and outbreaks of disease (Table 7). Isolates with PT 14a comprised the largest group of *E coli* O157:H7 strains in the year 2000. PTs 2 and 8 have been recently associated with outbreaks in nursing homes (Table 2). A total of 1353 *E coli* O157:H7 isolates were investigated using this extended phage typing scheme. Of these, 72.6% would have been classified as PT 14 based on pre-extended scheme criteria. However, after subdividing PT 14, these were separated into PT 14 (26.6%) and PT 14a (46%). Since 2000, the extended scheme has been used to subdivide 18 family outbreaks to give three that were due to PT 14 and 15 to PT 14a, while of 12 community outbreaks, four were identified as PT 14 and eight as PT 14a.

TABLE 3
Verotoxigenic *Escherichia coli* (VTEC) serotypes isolated from human sources in Canada between 1983 and 2000

Serotypes		
O1:H7	O80:H–	O121:H19
O2:H29	O82:H8	O126:H8
O5:H–	O84:H2	O128:H2, H12
O7:H4	O91:H–, H14, H21	O129:H7
O8:H2	O100:H10	O132:H–
O15:H27	O103: H–, H2, H11, H25	O145:H–
O16:H6	O104:H7	O146:H21
O26:H–, H11	O110:H–	O153:H11, H25
O38:H21	O111:H–, H8, H34	O157:H–, H7
O45:H2	O113:H21	O165:H–
O70:H11	O114:H21	O181:H49
O77:H18	O118:H30	O(Untypeable): H8,H25

Molecular typing using PFGE provided DNA ‘fingerprinting’, and this was highly sensitive for differentiating strains of both *E coli* O157:H7 and non-O157:H7 VTEC. A comprehensive review of PFGE typing in Canada is outside the scope of the present paper; however, examples that show the utility of the method for laboratory epidemiology are summarized briefly. PFGE of non-O157:H7 VTEC

TABLE 4
Verotoxigenic *Escherichia coli* serotypes isolated from cattle and meat sources in Canada between 1983 and 2000

Serotypes			
O1:H20, H7	O98:H-, H16, H25	O45:H2*	O138:H–, H14
O2:H-, H27, H29	O100:H–	O46:H–, H38	O139:H–, H1, H19
O5:H–*	O103:H2*, H8, H25		O141:H4
O6:H34	O110:H8	O69:H11	O142:H38
O7:H4, H7	O111:H–, H8, H11*	O74:H†	O145:H–, H8, H16*
O8:H2*, H9, H14, H16	O113:H–, H4*, H7, H21*	O75:H1, H8	O153:H–, H21, H25*, H31
O15:H–, H21, H27*	O115:H8, H18	O76:H†, H19, H21, H25	O156:H–, H8, H25
O18:H11	O116:H–, H21	O77:H39	O157:H–, H7*
O21:H21	O117:H–, H4	O80:H–*	O159:H49
O22:H8, H16	O118:H–, H16*	O82:H8*	O163:H–, H2, H19
O26:H–, H11*	O121:H7, H19*	O84:H–, H†, H25	O165:H–*
O35:H27	O126:H8, H27*	O85:H–	O171:H2
O38:H21*	O128:H35*	O88:H25, H49	O172:H–*
O40:H8	O132:H–*	O91:H–, H21	O(Untypeable):H–, H2, H8, H19, H21, H25, H34
O43:H2	O136:H–, H12, H16		

**Also identified worldwide among human strains; †H (untypeable)*

showed that most serotypes could be analyzed using the Centers for Disease Control and Prevention PFGE methodology (Figure 2), although changes in run conditions may be required to optimize the results. Serotypes O26:H11 and O26:H– clustered on the dendrogram (Figure 3), as did isolates from serogroup O113:H21. However, isolates of O145:H– were present on widely separated branches, suggesting greater pattern heterogeneity within these serotypes. All O121:H19 serotypes that constituted a cluster of cases in British Columbia in 2000 had identical PFGE patterns. These results suggested that PFGE was useful for surveillance and outbreak analysis of non-O157:H7 VTEC. PFGE was particularly valuable in outbreak investigations. In the 1999 salami-associated outbreak in Alberta and British Columbia, isolates of *E coli* O157:H7 from salami (Figure 4, lanes 8, 9 and 12) exhibited the same genetic profile as isolates from humans (Figure 4, lanes 3, 4, 6 and 7). This established a direct link between the salami and human illness.

DISCUSSION

The use of surveillance data and laboratory-based epidemiological markers by the NLEP has generated valuable information to support outbreak investigations of VTEC across Canada. Indeed, surveillance data at the NLEP from 1986 to 2000 have identified approximately 22,182 laboratory confirmed cases of *E coli* O157:H7. On average, this would represent 1478 cases annually. However, many outbreaks and sporadic cases go unreported, and to obtain a clearer understanding of the magnitude of the problem, it is necessary to determine the difference between the number of reported cases and the number of cases that actually occur in the community (37). The degree of under-reporting of *E coli* O157:H7, a pathogen that typically causes bloody diarrhea, has been estimated in the United States to be approximately 20-fold (37). Estimates for the province of Ontario suggest that 78% to 88% of symptomatic *E coli* O157:H7 cases in that province are missed by the surveillance system (38), although almost 100% of cases that were confirmed microbiologically were reported to the Ontario Ministry of Health. No estimates were provided for the subsequent reporting of cases to the NLEP; this is likely to be very close to 100% as well. However, the proportion of these isolates provided to, and analyzed at, the NLEP is not known. As a consequence, the total number of estimated cases of *E coli* O157:H7 in Canada each year may be closer to 30,000 and may result in approximately 15 to 25 deaths/year.

Mortality rates following infection with VTEC and associated HUS in developed countries are approximately 2% to 10% (30). One major outbreak in Canada in 2000 included more than 1000 reported cases of human illness and six deaths, and was associated with the consumption of contaminated water (35). Cattle represent the single most common reservoir for O157 and non-O157 VTEC strains causing infections in humans as a result of foodborne and waterborne fecal contamination. In the United States, food animals generate more than 1.6 billion tons of manure/year and the disposal of this material has become a growing problem in recent years (7). Indeed, the presence of large volumes of farm animal manure in the environment creates a potential reservoir for enteric pathogens and poses a continuing risk for the dissemination of human enteric diseases.

As the result of studies at the NLEP in Canada, more than 45 non-O157 VTEC serotypes were identified from human cases of diarrheal disease; these serotypes were also

TABLE 5
Geographical distribution of verotoxigenic *Escherichia coli* serotypes from human sources in Canada investigated by the Canadian National Laboratory for Enteric Pathogens between 1983 and 2000

Serotype	BC	AB	SK	MB	ON	QC	NB	NS	NF	PEI	Total	Ranking*
O1:H7	4	7			7			1	1		20	6
O2:H29		1									1	
O5:H–	1	1			1	1					4	
O7:H4	1										1	
O8:H2						1					1	
O26:H–								9	2	1	12	8
O26:H11	8	19	2	19	17	3		22		5	95	2
O38:H21		1									1	
O45:H–			1								1	
O70:H11			1								1	
O77:H18					1	1					2	
O80:H–					1						1	
O82:H8		1									1	
O84:H2					1						1	
O91:H–	2			1		1					4	
O91:H14					2						2	
O91:H21					2	1					3	
O100:H10			1								1	
O103:H2	4	4		3	1						12	8
O103:H11				1							1	
O103:H25	1										1	
O103:H–						1					1	
O104:H7	1			1							2	
O110:H–	1										1	
O111:H–	1	2		1	6			1		2	13	7
O113:H21	3	1			5			1			10	10
O114:H21	2										2	
O118:H30					1						1	
O121:H19	5		2	3		1					11	9
O126:H8	1							17	3	6	27	5
O128:H2	1				2	1		3		1	8	
O128:H12	1		2	2		1		51	2	2	61	3
O132:H–		1	1		1						3	
O145:H–	3	2		1	1	1					8	
O146:H21	1										1	
O153:H11	1										1	
O153:H25					1						1	
O157:H7	693	2525	464	729	1852	521	160	178	55	164	7341	1
O157:H–	10	2	2		8	15		1			38	4
O181:H49	1										1	

**Ranking of the 10 most common serotypes detected. AB Alberta; BC British Columbia; MB Manitoba; NB New Brunswick; NF Newfoundland; NS Nova Scotia; ON Ontario; PEI Prince Edward Island; QC Quebec; SK Saskatchewan*

TABLE 6
Verotoxigenic (VT) genotypes of *Escherichia coli* O157:H7 identified by polymerase chain reaction, 1994 to 2000

Year	Isolates	VT1 (%)	VT2* (%)	VT1+VT2 (%)	Other VT[†] (%)
2000	1004	0.8	24.4	69.2	5.6
1999	407	1.0	4.2	88.2	6.6
1998	602	0	4.2	93.3	2.5
1997	376	1.6	2.9	91.8	3.7
1996	829	3.5	4.3	65.0	27.2
1995	444	1.1	13.1	80.4	5.4
1994	250	1.2	8.0	75.6	15.2
Average percentage		1.3	8.7	80.5	9.5

**The higher than usual percentage of VT2 in 2000 was attributable to the Walkerton outbreak; [†]VT-negative and VTvh-variant human toxins in combination with other toxins or alone*

TABLE 7
The five most common *Escherichia coli* O157:H7 phage types (PTs) identified by the Canadian National Laboratory for Enteric Pathogens from human isolates, 1996 to 2000

Rank	1996	1997	1998	1999	2000
1	14 (182)	14 (213)	14 (596)	14 (289)	14 (776)*
2	4 (42)	31 (20)	4 (46)	8 (27)	4 (63)
3	2 (40)	8 (13)	8 (30)	4 (20)	8 (43)
4	32 (23)	1 (12)	1 (20)	31 (12)	1 (25)
5	31 (22)	32 (11)	31 (18)	32 (10)	31 (21)

**450 were PT 14a and 326 were PT 14; Expanded phage typing scheme placed into use in 2000. Numbers in parentheses indicate the number of isolates investigated*

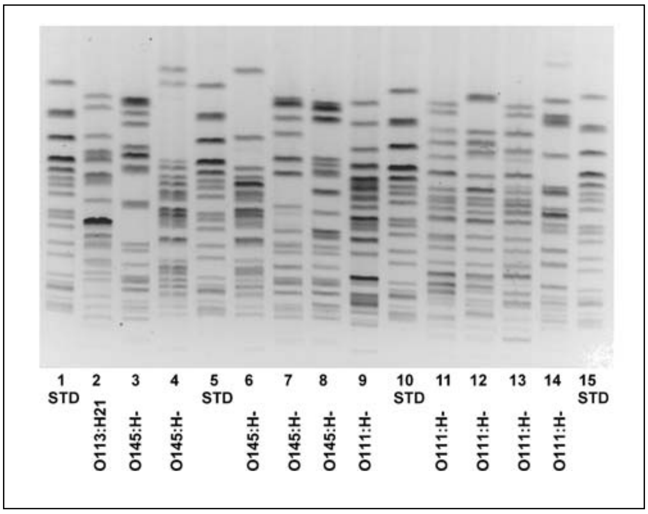

Figure 2) *Pulsed-field gel electrophoresis (PFGE) of selected non-O157 verotoxin* Escherichia coli *serotypes found in Canada. Lanes 1, 5, 10 and 15 contain PFGE standard strain G5244. Serotypes of each isolate tested are included below the lane number*

represented among the more than 100 serotypes identified in cattle and meat products. An increasing number of non-O157 VTEC serotypes have been isolated from humans with diarrheal disease, and as many as one in five cases of classic HUS may be due to VTEC other than *E coli* O157:H7 (36). Diarrheal disease associated with outbreaks and sporadic cases of the non-O157:H7 VTEC serotype, O111:H– has been reported in Australia, Germany, Italy and the United States. In addition, outbreaks attributable to other serotypes have been detected in Montana (O104:H21), Chile (O26:H11), Argentina (O18:H31) and France (O103:H2). Although not associated with large outbreaks in Canada, a variety of serotypes of non-O157 VTEC has been associated with numerous sporadic cases of human disease. Because many laboratories do not routinely check for VTs, and only reference laboratories do complete serotyping, the number of laboratory-confirmed cases of non-O157 VTEC is relatively low compared with the presumed incidence. However, it was intriguing to note that a large majority of the O126 and O128 isolates came from the maritime provinces of Nova Scotia, Newfoundland and Prince Edward Island. Indeed, the only other isolate of O126 that was recorded came from British Columbia, which is also a coastal province. The significance of a possible maritime link to these particular serotypes is not clear. Also of interest was the observation that the O128, O126 and O1 serotypes ranked higher than the O103 and O111 serotypes, both of which are quite prevalent in some countries other than Canada.

The significance of *E coli* O157:H7 and other VTEC serotypes as causative agents of diarrheal disease associated with severe complications such as hemorrhagic colitis, HUS and TTP is directly linked to the production of toxins, especially VTs, and other virulence factors (13). The production of VTs is mediated by lysogenic phages that code for the production of VTs – VT1 (Stx1) and VT2 (Stx2) (4,13). Among VTEC, the recognized toxin genotypes include VT2vh-a, VT2vh-b, VT2d, VT2ev and VT2e in combination with other toxins or found alone (33,34,39-41). In a recent waterborne outbreak in Canada, the *E coli* O157:H7 outbreak strain was identified as toxin genotype *Stx*2. During the seven-year period from 1994 to 2000, a total of 80.5% of 3912 O157 VTEC isolates tested at the NLEP were toxin genotype VT1 plus VT2, while VT2 comprised only 8.7%. It is not known whether the toxin genotype had an influence on the ability of this strain to cause the outbreak, whether other virulence factors may have been important, or if the selection of this strain from the available population of *E coli* O157:H7 was due to chance. However, this toxin genotype became a useful marker during the outbreak to help define outbreak-related cases and to guide the epidemiological investigation.

Molecular typing using PFGE has proven to be particularly valuable in a number of major Canadian outbreak investigations to establish a link between food isolates and human cases of O157 VTEC infection. The outcome of multiprovincial outbreaks of disease has been positively affected by the recent creation and implementation of PulseNet North, a molecular network based on PFGE subtyping, the function of which is the rapid dissemination of laboratory-based typing data across Canada. Through the PulseNet North program, all participants are informed of the clonality of isolates circulating in the various regions of Canada. This has the effect of speeding up outbreak detection and facilitating intervention strategies. Surveillance

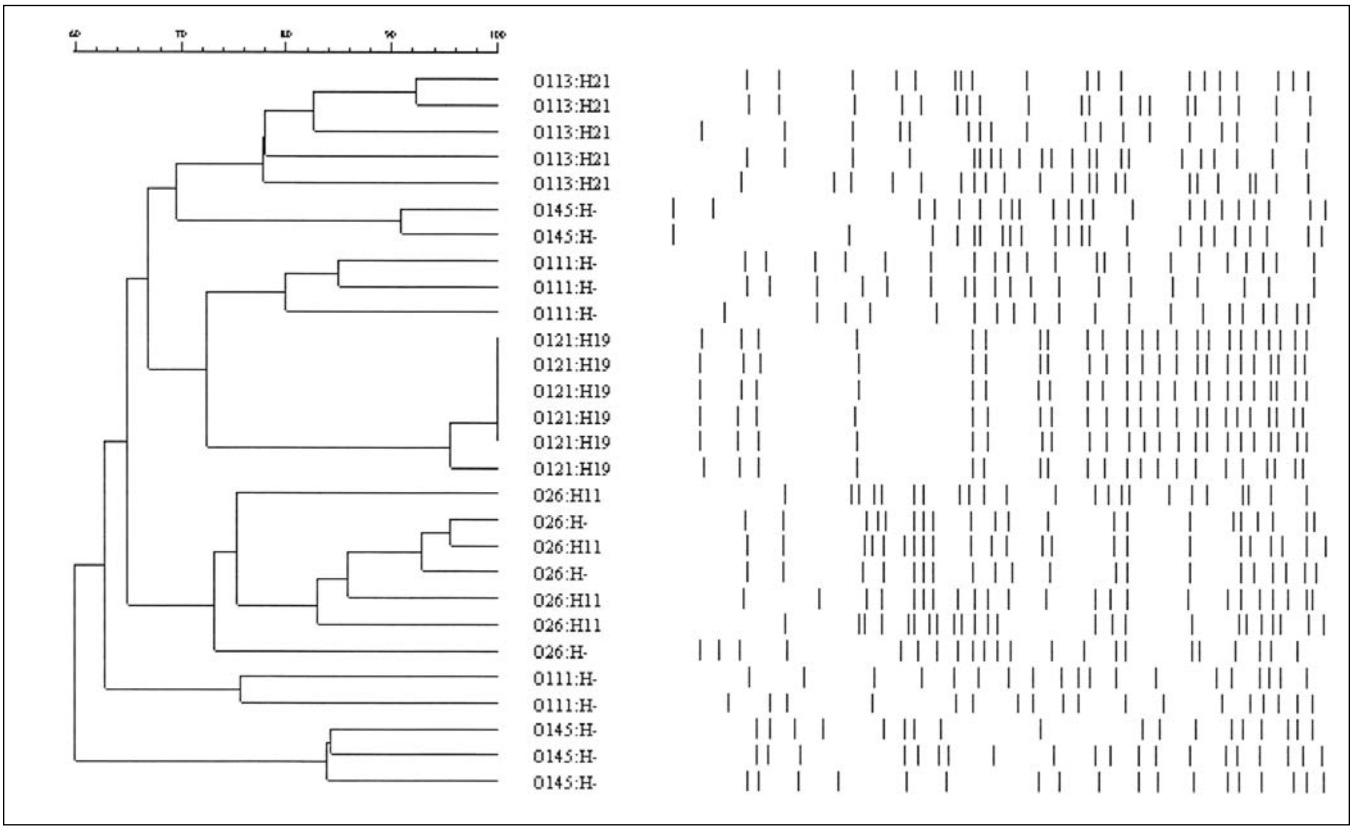

Figure 3) *Dendrogram distribution of non-O157 verotoxin* Escherichia coli *in Canada. Pulsed-field gel electrophoresis patterns were analyzed using Molecular Analyst 2.0 (Applied Maths Kortrijk, Belgium). The dendrogram was created using the Dice coefficient by the unweighted pair group method with arithmatic mean method, with a maximum band position tolerance of 1.5%*

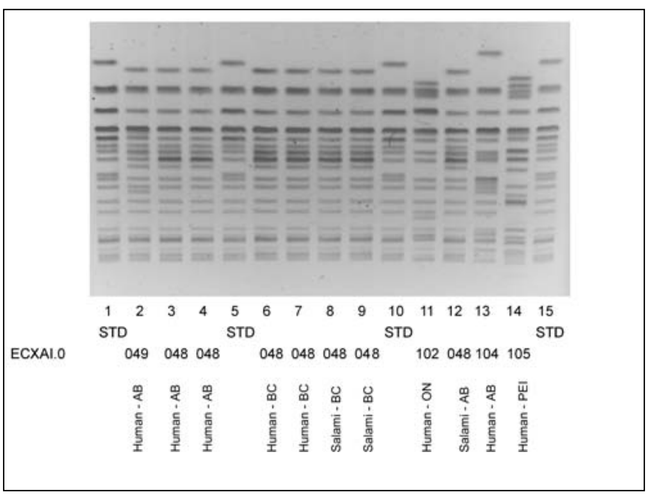

Figure 4) *Pulsed-field gel electrophoresis (PFGE) of the* Escherichia coli *O157:H7 outbreak associated with salami that affected three provinces in Canada. Lanes 1, 5, 10 and 15 contain PFGE standard strain G5244. PFGE pattern designations and sources of the strains are shown below each lane*

activities, along with epidemiological markers that include biotyping, serotyping, phage typing, toxin genotyping and molecular typing, represent the strongest link in characterizing foodborne and waterborne outbreaks of VTEC O157:H7 and tracing sources of human infection across Canada.

CONCLUSIONS

VTEC represents a serious public health threat in Canada in terms of the potential to cause life threatening human disease. Numerous sporadic cases and major outbreaks of disease have been associated with ground beef, unpasteurized milk, unpasteurized apple juice, salami, lettuce, alfalfa sprouts and untreated water.

Globalization of the food supply has increased the potential for outbreaks of VTEC from imported products (42). The NLEP is addressing VTEC as a high-priority health concern and is a major player in surveillance networks worldwide, including the PulseNet US, the Canadian PulseNet North and the European EnterNet, representing the national and international arena. Surveillance and epidemiological laboratory markers have strengthened our capabilities to detect the incidence of disease, to identify sources of infection, to define risk factors, to provide early detection of outbreaks and to determine the distribution and transmission of VTEC from food, water and animal sources to humans.

Prevention and control of *E coli* O157:H7 and other VTEC serotypes causing human illness is a high-priority concern for Canada and is driven by the high associated medical care costs, the loss of productivity, economic loss, and the increased morbidity and mortality associated with this condition. An increase in public awareness, along with education associated with safe food handling practices and concerted efforts by all levels of government and industry are required to prevent and control outbreaks of both *E coli* O157:H7 and other VTEC serotypes in Canada.

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